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European Reference Materials

CERTIFICATION REPORT

The certification of ^{202}Hg enriched methyl mercury amount content and isotopic composition Hg as methyl mercury in ethanol/water solution: ERM®-AE671

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Contact information
Reference materials sales
Address: Retieseweg 111, 2440 Geel, Belgium
E-mail: jrc-rm-distribution@ec.europa.eu
Tel.: +32 (0)14 571 705

JRC Science Hub
https://ec.europa.eu/jrc

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Abstract
This report describes the production of ERM®-AE671, a 2 % ethanol/water material certified for the amount content of isotopically enriched methyl mercury ($CH_3(^{202}Hg)$) and certified for the isotopic composition of Hg present as methyl mercury (CH_3Hg^+). The material was produced following ISO Guide 34:2009.
Twenty two ampoules of ERM-AE670 containing isotopically enriched methyl mercury chloride ($CH_3(^{202}Hg)Cl$) in 2 % ethanol/water solution were used as starting material. Each ampoule contained approximately 5 g of solution. The content of ERM-AE670 ampoules was gravimetrically diluted with 2 % ethanol/water solution to create enough material for 220 new units. The diluted $CH_3(^{202}Hg)Cl$ solution was filled into quartz ampoules which were flame sealed.
Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006.
The certified value was obtained from the gravimetric preparations, taking into account the amount of content and isotopic composition of the base material. The certified values were confirmed by isotope dilution mass spectrometry as independent verification method measurements within the scope of accreditation to ISO/IEC 17025:2005.
Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The main purpose of the material is to be used as spike isotopic reference material for determination of CH_3Hg content in an unknown samples by species-specific isotope dilution through a measurement of the mercury isotope amount ratio $R(B) = n(^{200}Hg)/n(^{202}Hg)$ of CH_3Hg, in a blend. The CRM is available in quartz glass ampoules containing 5 g of liquid species solution flame sealed under nitrogen atmosphere.
The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

The certification of ^{202}Hg enriched methyl mercury amount content and isotopic composition Hg as methyl mercury in ethanol/water solution: ERM®-AE671

M. Vahcic, J. Snell, C. Quétel, A. Held

European Commission, Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Geel, Belgium

Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM®-AE671, a 2 % ethanol/water material certified for the amount content of isotopically enriched methyl mercury ($CH_3(^{202}Hg)$) and certified for the isotopic composition of Hg present as methyl mercury (CH_3Hg^+). The material was produced following ISO Guide 34:2009 [1].

Twenty two ampoules of ERM-AE670 containing isotopically enriched methyl mercury chloride ($CH_3(^{202}Hg)Cl$) in 2 % ethanol/water solution were used as starting material. Each ampoule contained approximately 5 g of solution. The content of ERM-AE670 ampoules was gravimetrically diluted with 2 % ethanol/water solution to create enough material for 220 new units. The diluted $CH_3(^{202}Hg)Cl$ solution was filled into quartz ampoules which were flame sealed.

Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2].

The certified value was obtained from the gravimetric preparations, taking into account the amount of content and isotopic composition of the base material. The certified values were confirmed by isotope dilution mass spectrometry as independent verification method measurements within the scope of accreditation to ISO/IEC 17025:2005 [3].

Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The main purpose of the material is to be used as spike isotopic reference material for determination of CH_3Hg content in an unknown samples by species-specific isotope dilution through a measurement of the mercury isotope amount ratio $R(B) = n(^{200}Hg)/n(^{202}Hg)$ of CH_3Hg, in a blend. The CRM is available in quartz glass ampoules containing 5 g of liquid species solution flame sealed under nitrogen atmosphere.

The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

The following values were assigned:

	Amount content of $CH_3(^{202}Hg)$ and isotopic composition of Hg in CH_3Hg		
		Certified value [1]	Uncertainty [2]
Amount content	[mol $(CH_3(^{202}Hg)Cl \cdot g^{-1}]$ (solution)	$15.1 \cdot 10^{-9}$	$0.7 \cdot 10^{-9}$
Amount ratios of Hg isotopes in form of CH_3HgCl	$n(^{196}Hg)/n(^{202}Hg)$	0.000 018	0.000 013
	$n(^{198}Hg)/n(^{202}Hg)$	0.000 62	0.000 05
	$n(^{199}Hg)/n(^{202}Hg)$	0.001 60	0.000 10
	$n(^{200}Hg)/n(^{202}Hg)$	0.005 50	0.000 22
	$n(^{201}Hg)/n(^{202}Hg)$	0.013 4	0.000 6
	$n(^{204}Hg)/n(^{202}Hg)$	0.002 60	0.000 16

1) The values reported in this certificate result from gravimetric dilution and measurements performed at IRMM, and are traceable to the SI via the kg and the values of the isotopic reference material IRMM-639, which are traceable to the SI via the values of the Tl isotope ratios of the isotopic reference material NIST SRM 997.

2) The certified uncertainty is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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Glossary

ANOVA	Analysis of variance
c	Mass concentration $c = m / V$ (mass / volume)
CI	confidence interval
CRM	Certified reference material
EC	European Commission
EFSA	European Food and Safety Authority
ERM®	Trademark of European Reference Materials
EU	European Union
GC	Gas chromatography
GUM	Guide to the Expression of Uncertainty in Measurements [4]
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-QMS	ICP-Quadropole mass spectrometry
ICP-SFMS	ICP-Sector field mass spectrometry
ICP-MCMS	ICP-Multi collector mass spectrometry
ID	Isotope dilution
IDMS	Isotope dilution mass spectrometry
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LD_{50}	Lethal Dose, 50 %
LOD	Limit of detection
LOQ	Limit of quantification
LTS	Long-term stability study
M	Molar mass
MeHg	Methyl mercury
MS	Mass spectrometry
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable

n.c.	Not calculated
PTFE	Polytetrafluoroethylene
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RSD	Relative standard deviation
r^2	Coefficient of determination of the linear regression
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
se	Standard error
SI	International System of Units
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
STS	Short-term stability study
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
t_i	Time point for each replicate
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α and df degrees of freedom
t_{sl}	Proposed shelf life
u	standard uncertainty
U	expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	combined standard uncertainty; an additional index "rel" is added as appropriate
u_{cal}	Standard uncertainty of calibration
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate

u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
u_{t}	Standard uncertainty of trueness
VIM	Vocabulaire International de Métrologie – Concepts Fondamentaux et Généraux et Termes Associés (International Vocabulary of Metrology – Basic and General Concepts and Associated Terms) *[ISO/IEC Guide 99:2007]*
$\overline{x}$	Arithmetic mean
$\overline{x}_{ns}$	Arithmetic mean of all results of normal stock samples
$\overline{x}_{ref}$	Arithmetic mean of results of reference samples
α	significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Regulatory authorities are required to measure Hg in a variety of biological, industrial and food samples for reasons of public health. According to EU regulation (EC) No 466/2001: "Setting the maximum levels for contaminants in foodstuffs" and its amending annexes it is an obligation for EU member states to control concentrations of contaminants in foodstuffs. In addition to total Hg measurement, the specific measurement of methyl mercury (MeHg) is of interest to regulatory organizations; as this species has a mammalian lethal dose, 50 % (LD_{50}) 1000 times lower than elemental Hg. Many foodstuffs, particularly fish contain the majority of Hg as MeHg [5]. The 2012 recommendation from the European Food and Safety Authority (EFSA) panel on contaminants in the food chain states that the provisionally tolerable human consumption of MeHg is limited to 1.3 µg kg^{-1} body mass per week [6]. The current permitted wet weight concentration of Hg in fishery products (muscle meat of fish, muscle meat from appendages and abdomen of crustaceans) is up to 0.5 µg g^{-1} and up to 1.0 µg g^{-1} for certain species of fish listed in the regulation. Because the greater proportion of Hg can be present as MeHg, a fish consumer could easily exceed such a recommendation [5]. In order to meet the legislation requirements testing laboratories need to ensure the measurement results they produce are comparable and traceable to the same units of the same system of reference. This is most conveniently done through the use of an appropriate CRM that can be used for all types of samples without the need for matrix specific CRMs.

1.2 Choice of the material

A variety of approaches are available for the measurement of MeHg [7]. One of them is isotope dilution mass spectrometry (IDMS), an analytical technique capable of producing results with low uncertainty that are traceable to the SI. IDMS rests on the principle of measuring the change in the isotopic composition of the element measured from the addition of same isotopically enriched material to the sample of interest. For IDMS measurements to be species-specific, the species of the element in the added spike must be identical to that under scrutiny in the sample. Thus, attempting to apply IDMS to the measurement of MeHg requires a stable isotopically enriched MeHg spike [5].

To meet this requirement a CRM of isotopically enriched MeHg in solution, IRMM-670, was produced in 2003 and rebranded ERM-AE670 in 2004. When the original stock was close to exhaustion, it was decided to dilute the remaining 22 ampoules of ERM-AE670 approximately 10 times to produce 220 units of ERM-AE671, which still have MeHg content high enough for isotope dilution measurement of most samples.

ERM-AE671 is available in sealed quartz ampoules containing approximately 5 ml of isotopically enriched MeHg in 2 % ethanol/water solution with the amount content of 15.1 mol $(CH_3(^{202}Hg)Cl) \cdot g^{-1}$ and certified Hg isotopic ratios.

1.3 Design of the project

ERM-AE671 was characterised for the amount content of $CH_3(^{202}Hg)$ and certified isotopic composition of Hg present as CH_3Hg.

The MeHg content of ERM-AE671 was established by multiplication of the certified MeHg value of ERM-AE670 with the gravimetric dilution factor and verified by measurement of total Hg and inorganic Hg^{2+} content in ERM-AE671. For validation of the dilution, the MeHg content was measured by the same scheme as used in certification of ERM-AE670. This entailed subtraction of the inorganic Hg^{2+} content, measured specifically by gas

chromatography inductively coupled plasma mass spectrometry (GC-ICP-MS) with external calibration against dilutions of ERM-AE639 (natural Hg) [8] from the total Hg content, measured by ID-ICP-MS, applied as a primary method of measurement with ERM-AE639.

The material is intended to replace the ERM-AE670 spike isotopic reference material.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

2.3 Homogeneity study

ALS Scandinavia AB, Luleå, SE

2.4 Stability study

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

ALS Scandinavia AB, Luleå, SE

2.5 Characterisation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

3 Material processing and process control

3.1 Origin of the starting material

The starting material for ERM-AE671 comes from 22 ampoules of ERM-AE670 (IRMM-670), which was certified for the amount content of $CH_3(^{202}Hg)$ and isotopic composition of Hg present as CH_3Hg. Each ampoule contained approximately 5 g of solution. The solution matrix was 2 % ethanol in water [9].

Table 1: Amount content of $CH_3(^{202}Hg)$ in ERM-AE670 and isotopic composition of Hg present as CH_3Hg [9]

		Certified value	U (k=2)
Amount content	mol $(CH_3(^{202}Hg)Cl) \cdot g^{-1}$ (solution)	$171.0 \cdot 10^{-9}$	$6.1 \cdot 10^{-9}$
amount ratios of Hg isotopes in form of CH_3HgCl	$n(^{196}Hg)/n(^{202}Hg)$	0.000 018	0.000 013
	$n(^{198}Hg)/n(^{202}Hg)$	0.000 623	0.000 050
	$n(^{199}Hg)/n(^{202}Hg)$	0.001 603	0.000 096
	$n(^{200}Hg)/n(^{202}Hg)$	0.005 50	0.000 22
	$n(^{201}Hg)/n(^{202}Hg)$	0.013 35	0.000 53
	$n(^{204}Hg)/n(^{202}Hg)$	0.002 60	0.000 16

3.2 Processing

The production of ERM-AE671 was divided into several handling steps, the preparation of the dilution and the ampouling of the material.

Preparation of the dilution

The diluent was prepared by the addition of about 40 g ethanol to about 2 L of ultrapure water (18.2 MΩ·cm at 25 °C, Millipore corp, Billerica, USA). An empty 2 L PTFE bottle was weighed, about 1 L of diluent was transferred to the bottle, and the bottle was re-weighed. Ampoules of ERM-AE670 were brought to ambient temperature overnight and 21 ampoules were opened, the contents transferred to the bottle and the bottle was re-weighed. For buoyancy correction, the density of the diluent was measured (with ultrapure water as reference) using a Mettler density meter (tube vibration). Weighing of reagents used for gravimetric dilution is summarised in Table 2.

Table 2: Masses of solvent and ERM-AE671 dilute used for gravimetric dilution of ERM-AE670

Parameter	Mass [g]	U (k=2) [g]
Solvent	1068.96	0.02
ERM-AE671 dilute	1172.53	0.02

Ampouling of ERM-AE671

About 1.5 hours after preparation of the dilution, ERM-AE671 was transferred to ampoules on a clean bench using a peristaltic pump. The filling of ampoules was divided into two sessions of about 2 hours each, with 15 ampoules being filled with blank diluent solution at

the beginning of each session. At least 5 g of solution were transferred to each ampoule: ampoules were flushed with nitrogen and were immediately flame-sealed. Ampoules were then labelled and sealed in plastic bags. The complete batch was then transferred to -20 ºC freezers.

3.3 Process control

The control of the produced material was performed after processing on 10 randomly selected ampoules. The following analyses were made:

- The measurement of total Hg mass fraction by IDMS:
    - To confirm that the measured values and the value obtained from gravimetric dilution of ERM-AE670 agree within their respective uncertainties.
    - To calculate the MeHg mass fraction and to confirm that the calculated MeHg value and the MeHg value obtained by gravimetric dilution agree within their respective uncertainties.
    - To confirm that the isotopic composition of the new material and the isotopic composition of the ERM-AE670 agree within their respective uncertainties. Significant change in isotopic composition would indicate contamination with natural Hg.
- The measurement of inorganic Hg^{2+} mass fraction by IDMS:
    - To calculate the MeHg mass fraction and to confirm that the calculated MeHg value and the MeHg value obtained by gravimetric dilution agree within their respective uncertainties.
    - To compare the isotopic composition of the new material with the isotopic composition of ERM-AE670. Significant change in isotopic composition would indicate contamination with natural Hg.

The results of these tests are shown in Annex F.

4 Homogeneity

A key requirement for any reference material is the equivalence between the various units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value. In contrast to that it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity was not necessary because the material is a true solution and as such can be regarded completely homogenous.

4.1 Between-unit homogeneity of the MeHg amount content

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainty.

The number of selected units (10 units in total) is greater than the cubic root of the total number of the produced units (220 in total). The 10 units were selected using a random

stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 10 groups (with a similar number of units) and one unit was selected randomly from each group. Three independent samples were taken from each selected unit, and analysed by ICP-MS for inorganic Hg^{2+} and total Hg content. The measured content for inorganic Hg^{2+} was subtracted from the measured content for total Hg to calculate the MeHg content using equation 1. The calculated MeHg content was then used for calculation of homogeneity. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence.

$$\boldsymbol{C_{MeHg} = C_{Hg_{total}} - C_{Hg_{inorganic}}} \qquad \textbf{Equation 1}$$

c amount content [mol/g]

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence or the analytical sequence were visible.

The dataset was tested for consistency using Grubbs outlier tests on a confidence level of 99 % on the individual results and the unit means. No outlying individual result and outlying unit mean was detected.

Quantification of between-unit inhomogeneity was accomplished by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole unit.

Evaluation by ANOVA requires unit means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the unit means was visually tested using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The statistical evaluation of the MeHg homogeneity on 95 % confidence level showed no trends in analytical and filling sequences, no outliers and normal distribution of individual results and unit means.

One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [10]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 3}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}}$$ **Equation 4**

MS_{within}	mean square within a unit from an ANOVA
$MS_{between}$	mean squares between-unit from an ANOVA
$\bar{y}$	mean of all results of the homogeneity study
n	mean number of replicates per unit
$v_{MSwithin}$	degrees of freedom of MS_{within}

Table 3: Results of the homogeneity study

	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{bb,rel}$ [%]
Amount content MeHg	0.49	0.18	0.16	0.18

The homogeneity study showed no outlying unit mean or trend in the filling sequence or analytical sequence. Therefore the between-unit standard deviation can be used as estimate of u_{bb}. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-unit homogeneity and minimum sample intake of the MeHg amount content

The within-unit homogeneity is closely correlated to the minimum sample intake. Due to this correlation, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

Measures were taken to avoid contamination with natural Hg during processing. This assumption was confirmed by the stability study, where sample intakes as low as 0.14 g were found to give acceptable repeatability, demonstrating that there is no intrinsic inhomogeneity or contamination at a sample intake of 0.14 g. However as the material is a true solution no minimum sample intake should be required, however the minimum sample intake was set to 10 mg as this is the minimum amount of sample that can be practically weighed with acceptable uncertainty for IDMS.

4.3 Homogeneity of the isotope ratios

The material was processed by gravimetric dilution of ERM-AE670. Any difference from the isotope amount ratios compared to ERM-AE670 can only stem from contamination with MeHg or inorganic Hg having a different isotopic composition than ERM-AE670.

'As the material was processed in a clean laboratory in which no materials containing MeHg of natural isotopic composition were kept, contamination with MeHg can be ruled out.

The amount content of inorganic Hg in ERM-AE670 was measured as $3.47 \cdot 10^{-9}$ mol·g^{-1} with an expanded uncertainty (k=2) of $0.71 \cdot 10^{-9}$ mol·g^{-1}.

Inorganic Hg in ERM-AE671 was measured in 9 vials (single measurement per vial) with an average of $1.08 \cdot 10^{-10}$ mol·g^{-1} with an expanded uncertainty of $1.1 \cdot 10^{-11}$ mol·g^{-1}. This is a

factor 140 below the amount content of MeHg. The relative standard deviation of the 9 measurements was 11.2 %.

The maximum contribution of inhomogeneity of the inorganic Hg to the isotope ratios of MeHg is therefore 11.2 %/140 = 0.08 %. Moreover, an analysis of the isotope ratios in ERM-AE671 (one vial only) also showed that that isotope ratios of inorganic Hg does not differ from the isotope ratios of the total Hg.

From these considerations it is concluded that the homogeneity contribution to the uncertainty of the isotope ratios is negligible.

5 Stability

Time, temperature and radiation were regarded as the most relevant influences on stability of the materials. The influence of ultraviolet or visible radiation was minimised by the choice of storage and shipping conditions. The containment material (quartz ampoule) was chosen to prevent adsorption of Hg on inner surfaces of the containment vessel. Since the materials are to be stored and dispatched in the dark which practically eliminates the possibility of degradation by radiation, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies were carried out using an isochronous design [11] for the short-term stability study (STS) and modified standard design for the long-term stability (LTS). In isochronous design approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests. For the LTS, a modified standard design based on the certification data of ERM-AE670 and certification data of ERM-AE671 was used. The ERM-AE670 and ERM-AE671 data was combined because ERM-AE671 is expected to have the same stability properties as ERM-AE670, as the matrix (diluent) and container material are the same, and both series of ampoules were filled under nitrogen.

5.1 Short-term stability study of the MeHg amount content

For the short-term stability study, samples were stored at 60 °C for 0, 1 and 2 weeks. The reference temperature was set to -20 °C. One unit per storage time was selected using a random stratified sampling scheme. From each unit, one sample was measured by GC-ICP-MS. The measurements were performed under repeatability conditions, and in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time.

The results were screened for outliers using the single and double Grubbs test. No outlying individual results were found.

Furthermore, the data were evaluated against storage time and regression lines of relative mass fraction versus time were calculated. The slope of the regression line was tested for statistical significance (loss/increase due to shipping conditions). For MeHg, the slope of the regression line was not significantly different from zero (on 95 % confidence level) at 60 °C.

The results of the measurements are shown in Annex C.

The material can be dispatched without further precautions under ambient conditions.

5.2 Long-term stability study of the MeHg amount content

The long term stability was assessed by comparing the MeHg data to the gravimetric dilution of ERM-AE670. The LTS combines the data from ERM-AE670 and ERM-AE671 studies. ERM-AE671 samples were stored at -20 °C for 47 and 139 months relative to the characterisation date of ERM-AE670. 6 samples at 0 month, 9 samples at 47 months and 10 samples at 139 months relative to the characterisation date of ERM-AE670 were selected using a random stratified sampling scheme for inorganic Hg^{2+} measurement. Inorganic Hg^{2+} was measured in one sample from each unit by GC-ICP-MS at all-time points except at the last where it was measured by ion exchange column separation-ICP-MS. The reason for the method change was that the subcontracting laboratory's attempts to detect inorganic Hg^{2+} with GC-ICP-MS method were unsuccessful. Inorganic Hg^{2+} was used for calculation of the MeHg contents using the Equation 1. The measurements at each time point were performed under repeatability conditions.

The presence of DMeHg was discounted by the fact that no detectable DMeHg was present in the original ERM-AE670 material from which the ERM-AE671 was prepared by gravimetric dilution. The measurements on the ERM-AE670 material in 2003 showed no detectable DMeHg, which proved that no DMeHg was present in the ERM-AE670 above the detection limit [12]. Because ERM-AE671 was produced from ERM-AE670 by approximately 10 times dilution and because there is still some presence of inHg^{2+} in the new solution it is unlikely that the formation of DMeHg would take place as the stability constants favour the formation of MeHg over DMeHg in the presence of inHg^{2+} [13]. Additionally no peaks for DMeHg were found in the analyses made in 2007 and no DMeHg peaks were reported by subcontracting laboratory performing the analyses in 2015. The presence of DMeHg in ERM-AE671 in significant quantities this would be reflected by the lowered mass fraction of MeHg, which was proved not to be the case.

Because the data for the stability test model comes from both ERM-AE670 and ERM-AE671 studies and because the MeHg amount contents are different in the two materials, the ERM-AE670 LTS data for MeHg content was multiplied by gravimetric dilution factor in order to enable comparison of MeHg data from ERM-AE670 and ERM-671 studies.

This approach was used because in contrast to the STS study the LTS study was not designed as isochronous study and the direct MeHg measurement method used in STS study proved to have insufficiently good repeatability to evaluate u_{lts} with the low level of uncertainty desired. Additionally there is a lack of MeHg calibrants for direct MeHg measurement methods that could be considered sufficiently traceable to make comparable measurements years apart. The MeHg contents in the ERM-AE670 samples were calculated using the ERM-AE670 certified values for measured total Hg, and inorganic Hg^{2+}. For ERM-AE671 samples the MeHg contents were calculated using the total Hg value obtained by gravimetric dilution of ERM-AE670 and measured inorganic Hg^{2+} values.

The results were screened for outliers using the single and double Grubbs test. No outlying individual results were found.

Furthermore, the combined data were plotted against storage time and linear regression lines of equivalent MeHg concentrations. The slope of the regression lines was tested for statistical significance (loss/increase due to storage conditions). For MeHg, the slope of the regression line was significantly different from zero (on 95 % and 99 % confidence levels).

A positive trend was observed for MeHg in the long-term standard stability study. As the analyte cannot be created in the sample, a positive trend could be due to different measurement methods used for inorganic Hg^{2+} in the last time point or due to loses during the analysis procedure (small amount of inorganic Hg^{2+} present in the material). However the measured and gravimetric total Hg mass fractions agree within their uncertainties (annex F) which indicate the total Hg mass fraction remains unchanged. The observed trend was therefore regarded as measurement method artefact but was nevertheless included in the

$u_{lts,\ rel}$ calculation in order to ensure a more robust estimation of LTS. The material can therefore be stored at -20 °C.

5.3 Estimation of uncertainties of the MeHg amount content

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [14] for MeHg amount content. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated for $u_{sts,rel}$ and the uncertainty of the linear regression line with a slope is calculated for $u_{lts,rel}$. The uncertainty contributions u_{sts} and u_{lts} are calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{tt} \qquad \textbf{Equation 5}$$

$$u_{lts,rel} = \left(\sqrt{\left(\frac{b \cdot t_{sl}}{\sqrt{3}} \right)^2 + u_b^2} \right) \cdot t_{sl} \qquad \textbf{Equation 6}$$

RSD	relative standard deviation of all results of the stability study
x_i	result at time point *i*
$\bar{x}$	mean results for all time points
t_{tt}	chosen transport time (1 week at 60 ºC)
t_{sl}	chosen shelf life (24 months at -20 ºC)
b	slope of the regression line
u_b	Standard error of the slope of the regression line

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 60 °C studies. The uncertainty describes the possible change during a dispatch at 60 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the -20 °C study. The uncertainty contribution describes the possible degradation during 24 months storage at -20 °C.

The results of these evaluations are summarised in Table 4.

Table 4: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of -20 °C and 2 years

	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
Amount content MeHg	1.14	0.22

No significant degradation during dispatch even at 60 °C was observed. Therefore, the material can be transported at ambient conditions without special precautions.

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

Higher $u_{sts,rel}$ was due to two factors. The first one was the method used (GC-ICP-MS) for determination of MeHg and the second one was the length of the STS. Because GC-ICP-MS method was used for determination of MeHg the measurement uncertainty of the method significantly influenced the $u_{sts,rel}$ of the STS. Additionally the short term stability was tested only for 0, 1 and 2 weeks which additionally increased predicted $u_{sts,rel}$. Because of the low stock of the ERM-AE671, it was decided that the STS study should not be repeated in 2015 to try to improve the $u_{sts,rel}$ for the STS.

5.4 Stability of the Hg isotope amount ratios

As is the case for homogeneity, a change of the isotope ratios can only be caused by an exchange of Hg with Hg derived from contamination by MeHg or inorganic Hg with a different isotopic composition from ERM-AE670.

The measured value of inorganic Hg in ERM-AE671 is even slightly lower than the value of ERM-AE670, when taking into consideration the dilution and is a factor 150 below the amount content of ERM-AE671(see section 4.30).

As the total amount of inorganic Hg that could exchange with MeHg is very low and as the inorganic Hg has the same isotopic composition of the total Hg, it is concluded that potential degradation has no effect on the isotope ratios. The uncertainty contribution of transport and storage for the isotope ratios is therefore negligible.

6 Characterisation

The material characterisation is the process of determining the property values of a reference material.

The material characterisation was based on a primary method of measurement, confirmed by an independent analysis. A primary method of measurement (also called "primary reference method" in the International Vocabulary of Metrology (VIM) [15]) is a method that does not require calibration with a standard of the same measurand and does not depend on a chemical reaction. Such methods are of highest metrological order and often yield results with low uncertainties. However, it is nevertheless prudent to demonstrate absence of bias or gross errors by use of an independent method of lower metrological order.

6.1 Purity of the base material

The purity of the base material was determined in the certification study for ERM-AE670 [12].

6.2 Mass fraction and their uncertainties

The MeHg amount content of ERM-AE671 was established by multiplication of the certified MeHg value of ERM-AE670 with the gravimetric dilution factor.

Characterisation was done in-house complying with the ISO/IEC 17025 requirements for testing, calibration and sample preparation. The data evaluation was done according to ISO Guide 34.

The value generated for the MeHg content in ERM-AE671 is based on multiplication of the certified value for the MeHg in ERM-AE670 with the gravimetric dilution factor. The total content of MeHg in ERM-AE671 from which all certified amount contents and mass fractions are derived was calculated using the following equation:

$$C_{MeHg_{(AE671)}} = C_{MeHg_{(AE670)}} \cdot \left(\frac{m_{total(AE671)} - m_{dilutent(AE671)}}{m_{total(AE671)}}\right)$$ **Equation 7**

c	amount content [mol/g]
m	mass
$m_{total(AE671)}$	mass of dilutes ERM-AE671 [g]
$m_{dilueent(AE671)}$	mass of solvent [g]

Table 5: Uncertainty of characterisation for amount content of MeHg

ERM-AE671	Mean [mol/g]	u_{char} [mol/g]
Amount content CH_3HgCl	$15.47 \cdot 10^{-9}$	$0.27 \cdot 10^{-9}$
Amount content $CH_3(^{202}Hg)Cl$	$15.11 \cdot 10^{-9}$	$0.27 \cdot 10^{-9}$

6.3 Verification measurements

The gravimetric dilution was verified by subtraction of measured inorganic Hg^{2+} content in ERM-AE671 from gravimetric total Hg value for ERM-AE671.

The verification measurements resulted in one dataset per MeHg, total Hg and inorganic Hg^{2+}. All individual results grouped per MeHg, total Hg and inorganic Hg^{2+} are displayed in tabular form in Annex F.

An assessment of the blank Hg content of the diluent and all containers used in processing was also made to confirm there was no significant contamination with natural Hg which could change the isotopic composition after dilution. The measurements showed that the content of the ampoules were about 10^{-6} times the mass fractions of Hg in ERM-AE671. The table with the results is displayed in tabular form in Annex F (Table F6).

In 2015 additional analyses were made to verify the stability of Hg isotope ratios in MeHg and inorganic Hg^{2+} (present as impurity). The isotopic composition of inorganic Hg^{2+} and isotopic composition of Hg in MeHg were verified by measuring Hg isotopic abundances in inorganic Hg^{2+} and MeHg in one ampoule using ion-exchange-ICP-SFMS to make sure the isotopic composition did not change during the years of storage or that there was no contamination. The results are displayed in tabular form in Annex F.

Verification of the gravimetric dilution and isotopic composition of ERM-AE670 showed that the gravimetric and measured values for total Hg agree within their uncertainties (Annex F). The measurements done to verify the level of Hg introduced from the diluent and all of the containers used in the processing showed the Hg level was too low to alter isotope ratios by a measurable amount and could not alter the isotopic ratios by more than the uncertainties assigned to ERM-AE670. The signals for all isotopes except ^{202}Hg were too low to quantify reliably and measured isotope ratios showed no difference from those certified for ERM-AE670, albeit within an uncertainty of measurement greater than that certified. It is a reliable assumption that any Hg contamination of the reagent and containers used in the preparation of ERM-AE671 and the ampoules in which the material was filled, would be inorganic and of natural isotopic composition.Through the measurement of the inorganic Hg^{2+} isotopic composition and total Hg procedural blank content it was shown that no Hg speciesis present in ERM-AE671 that could alter the isotope ratios relative to those certified in ERM-AE670, even in the case that methyl- groups would be exchanged between Hg species over time. The re-measured ratios of Hg in MeHg are shown in Table 6.

The verification measurements for total Hg, MeHg and inorganic Hg^{2+} show that the isotopic ratios in the ERM-AE671 remain the same as in the ERM-AE670 after gravimetric dilution of ERM-AE670.

Table 6: Measured isotopic composition of Hg present as CH_3HgCl.

	Measured value	U (k=2)
$n(^{196}Hg)/n(^{202}Hg)$	0.00003	0.00009
$n(^{198}Hg)/n(^{202}Hg)$	0.0007	0.0002
$n(^{199}Hg)/n(^{202}Hg)$	0.0016	0.0001
$n(^{200}Hg)/n(^{202}Hg)$	0.0056	0.0006
$n(^{201}Hg)/n(^{202}Hg)$	0.0136	0.0008
$n(^{204}Hg)/n(^{202}Hg)$	0.0027	0.0002

6.4 Methods used

The inorganic Hg^{2+} content was measured by GC-ICP-MS, with external calibration against dilutions of ERM-AE639 Hg in 0.5 M HCl.

The total Hg content and was measured by ID-ICP-MS with ERM-AE639 Hg in 0.5 M HCl used as a spike material.

The total Hg isotopic abundances were measured by ICP-MCMS.

Hg isotopic abundances in MeHg were measured by ion exchange separation ICP-SFMS.

Verification of isotopic composition of the inorganic Hg^{2+} was made by measuring the inorganic Hg^{2+} by ion exchange separation ICP-SFMS.

All methods used during the characterisation study are summarised in Annex E.

7 Value Assignment

Certified values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

In addition, derived values are reported as described in the following section.

7.1 Certified values and their uncertainties

The certified MeHg value is based on value obtained by multiplication of the certified value for the MeHg in ERM-AE670 with the gravimetric dilution factor.

Isotopic composition of Hg in CH_3Hg in ERM-AE671 is based on isotopic composition of ERM-AE670 from which it was produced by gravimetric dilution and the assessment of blank Hg content of the diluent and all containers used in processing.

The assigned uncertainties for isotopic amount ratios for ERM-AE670 and consequently for ERM-AE671 consists of certified ERM-AE640 (IRMM-640) [16] ratio uncertainties and the difference between the measured values for MeHg and the ERM-AE640 certified values, and

the uncertainties estimated by propagation of estimation on the MeHg ratio measurement uncertainties, as described in the ERM-AE670 certification report.

The certified isotopic amount ratios of Hg in the form of CH_3HgCl and their uncertainties come from ERM-AE670 certificate of analysis and are summarised in Table 7.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5). These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM, rel}$) with a coverage factor *k* as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{char,rel}^2 + u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2} \quad \textbf{Equation 8}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{sts} was estimated as described in section 5.3.
- u_{lts} was estimated as described in Section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor *k* of 2 was applied, to obtain the expanded uncertainties.

The certified values and their uncertainties are summarised in Table 8.

Table 7: Certified isotopic amount ratios of Hg in the form of CH_3HgCl and their uncertainties. Source: ERM-AE670 certificate of analysis

ERM-AE671	Amount ratios of Hg isotopes in the form of CH_3HgCl	Certified value	Uncertainty[1)]
	$n(^{196}Hg)/n(^{202}Hg)$	0.000 018	0.000 013
	$n(^{198}Hg)/n(^{202}Hg)$	0.000 62	0.000 05
	$n(^{199}Hg)/n(^{202}Hg)$	0.001 6	0.000 10
	$n(^{200}Hg)/n(^{202}Hg)$	0.005 50	0.000 22
	$n(^{201}Hg)/n(^{202}Hg)$	0.013 4	0.000 6
	$n(^{204}Hg)/n(^{202}Hg)$	0.002 60	0.000 16

[1)] Expanded (*k* = 2) and rounded uncertainty.

Table 8: Certified values and their uncertainties for ERM-AE671

ERM-AE671	Certified value [mol/g]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [mol/g] [1)]
Amount content CH_3HgCl	$15.5 \cdot 10^{-9}$ mol/g [1)]	1.77	0.18	1.14	0.22	4.25	$0.7 \cdot 10^{-9}$
Amount content $CH_3(^{202}Hg)Cl$	$15.1 \cdot 10^{-9}$ mol/g [1)]	1.77	0.18	1.14	0.22	4.25	$0.7 \cdot 10^{-9}$

[1)] Expanded (*k* = 2) and rounded uncertainty.

Derived values for the amount and mass contents, the isotopic composition of Hg in the form of CH_3HgCl and the molar mass of Hg in the form of CH_3HgCl were calculated by using CH_3Hg certified values, Hg atomic masses (Table 10) and certified amount ratios of Hg isotopes in MeHg. Because the derived values were calculated from the certified values using well understood mathematical model they are also considered certified values. The calculated certified values and their uncertainties are summarised in Table 9.

Table 9: Certified values: amount and mass contents, the isotopic composition of Hg in the form of CH_3HgCl and the molar mass of Hg in the form of CH_3HgCl

ERM-AE671		Certified value	Uncertainty[1)]
Amount content CH_3HgCl	mol (CH_3HgCl) · g^{-1} (solution)	$15.5 \cdot 10^{-9}$	$0.7 \cdot 10^{-9}$
Mass fractions	g (^{202}Hg as CH_3HgCl) · g^{-1} (solution)	$3.05 \cdot 10^{-6}$	$0.13 \cdot 10^{-6}$
	g (Hg as CH_3HgCl) · g^{-1} (solution)	$3.12 \cdot 10^{-6}$	$0.14 \cdot 10^{-6}$
Isotope amount fractions of Hg in the form of CH_3HgCl ·(100)	n(^{196}Hg)/n(Hg) n(^{198}Hg)/n(Hg) n(^{199}Hg)/n(Hg) n(^{200}Hg)/n(Hg) n(^{201}Hg)/n(Hg) n(^{202}Hg)/n(Hg) n(^{204}Hg)/n(Hg)	0.001 8 0.061 0.156 6 0.537 1.30 97.69 0.254	0.001 3 0.005 0.001 0 0.021 0.05 0.06 0.016
Isotope mass fractions of Hg in the form of CH_3HgCl ·(100)	m(^{196}Hg)/m(Hg) m(^{198}Hg)/m(Hg) m(^{199}Hg)/m(Hg) m(^{200}Hg)/m(Hg) m(^{201}Hg)/m(Hg) m(^{202}Hg)/m(Hg) m(^{204}Hg)/m(Hg)	0.001 7 0.060 0.154 3 0.532 1.30 97.70 0.257	0.001 2 0.005 0.0010 0.021 0.06 0.06 0.016
Molar mass of Hg in the form of CH_3HgCl	g · mol^{-1}	201.944 7	0.000 8

[1)] Expanded (k = 2) and rounded uncertainty.

Table 10: Atomic masses used for calculation of the derived values [17]

Isotope	g · mol^{-1}	Uncertainty (k=2)
^{196}Hg	195.965 814	0.000 008
^{198}Hg	197.966 752	0.000 006
^{199}Hg	198.968 262	0.000 006
^{200}Hg	199.968 309	0.000 006
^{201}Hg	200.970 285	0.000 006
^{202}Hg	201.970 625	0.000 006
^{204}Hg	203.973 475	0.000 006

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

CH_3HgCl is a chemically clearly defined analyte/substance. Identity was confirmed by GC-ID-ICP-MS analysis. The measurand is therefore structurally defined and independent of the measurement method.

Quantity value

The certified value was obtained by gravimetric preparation from CRM ERM-AE670. The traceability chain is based on the use of calibrated balances and a thorough control of the weighing procedure. The values of ERM-AE671 are therefore traceable to the SI via the values of ERM-AE670.

Isotopic composition of Hg in CH_3Hg is traceable to the SI through the use of gravimetric dilution of ERM-AE670 and the assessment of blank Hg content of the diluent and all containers used in processing.Isotopic composition of Hg in CH_3Hg in ERM-AE670 is in turn traceable to ERM-AE640. Both ERM-AE670 and ERM-AE640 were produced from the same stock of enriched ^{202}Hg and the synthesised MeHg was proven to be without any evident contamination with $^{natural}Hg$ during ERM-AE670 certification.

8.2 Commutability

The ERM-AE671 was prepared by gravimetric dilution of CRM ERM-AE670. The analytical behaviour of the new material will be the same as that from which it was prepared (ERM-AE670). However, commutability is not relevant as the material is not made to approximate any particular sample matrix.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The materials shall be stored at -20 °C ± 5 °C in the dark. Care shall be taken to avoid change of the Hg isotopic composition once the units are open, as the material is prone to Hg contamination. The user is reminded to transfer the material into a clean closable and Hg contamination free container immediately after taking a sample or opening the ampoule.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened ampoules.

9.3 Preparation and use of the material/Reconstitution

Before use, the material must be allowed to reach ambient temperature. The ampoule should be cleaned with a dust-free wipe wetted with purified water before opening. The material is ready for use after ampoule opening.

9.4 Minimum sample intake

ERM-AE671 MeHg in 2 % ethanol/water is a true solution. The stability study measurements were performed with sample intake as low as 0.14 g proving the individual samples are homogenous at least to this level. As the material is a true solution no minimum sample intake should be required, however it was set to 10 mg as this is the minimum amount of sample that can be practically weighed with acceptable uncertainty for IDMS.

9.5 Use of the certified value

The main purpose of the material is to be used as spike isotopic reference material for determination of the MeHg content in an unknown samples by species-specific isotope dilution through a measurement of the mercury isotope amount ratio R(B) = n(^{200}Hg)/n(^{202}Hg) of MeHg, in a blend.

The CH_3Hg content in unknown samples should be calculated with the aid of the following equation, which enables an easy quantification of the uncertainty sources in the procedure:

$$c(Hg,X) = \frac{R(Y) - R(B)}{R(B) - R(X)} \cdot \frac{\sum R_i(X)}{\sum R_i(Y)} \cdot \frac{m(Y)}{m(X)} \cdot c(Hg,Y) \qquad \text{Equation 9}$$

$R(X)$ = amount ratio n(^{200}Hg)/n(^{202}Hg) in the unknown sample material X

$R(Y)$ = amount ratio n(^{200}Hg)/n(^{202}Hg) in the spike material Y

$R(B)$ = amount ratio n(^{200}Hg)/n(^{202}Hg) in the blend B

$\sum R_i(X)$ = sum of all amount ratios in the unknown sample material X

$\sum R_i(Y)$ = sum of all amount ratios in the spike material Y

$m(X)$ = mass of unknown sample used in the measurement

$m(Y)$ = mass of the sample spike solution used in the measurement

$c(Hg,X)$ = amount content of the Hg $\cdot$ g^{-1} sample material

$c(Hg,Y)$ = amount content of the Hg $\cdot$ g^{-1} spike solution

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11 References

1 ISO Guide 34, General requirements for the competence of reference materials producers, International Organization for Standardization, Geneva, Switzerland, 2009

2 ISO Guide 35, Reference materials – General and statistical principles for certification, International Organization for Standardization, Geneva, Switzerland, 2006

3 ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories, International Organization for Standardization, Geneva, Switzerland, 2005

4 ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement, (GUM 1995), International Organization for Standardization, Geneva, Switzerland, 2008

5 J. Snell, C. Quetel, L. Lambertson, J. Qvarnstrom, Preparation and certification of ERM-AE670, a ^{202}Hg enriched methylmercury isotopic reference material, JAAS, 2004, 19, 1315- 1324.

6 Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food, EFSA Journal 2012;10(12):298

7 ‘‘Special issue: Sources of Error in Methylmercury Determination During Sample Preparation, Derivatisation and Detection,’’ Chemosphere, ed. R. Falter, 1999, vol. 39, no. 7, pp. 1037–1224.

8 Certificate of analysis ERM®-AE639

9 Certificate of analysis ERM®-AE670

10 T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6 (2001) 20-25

11 A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360 (1998) 359-361

12 J.Snell, C. Quétel, Certification of ^{202}Hg enriched methylmercury amount content and isotopic composition in candidate isotopic reference material IRMM-670, GE/R/IM/42/03, 2003

13 L. Bytautas, Stability of Dimethylmercury and Related Mercury-containing Compounds with Respect to Selected Chemical Species Found in Aqueous Environment, Croat. Chem. Acta 86 (4) (2013) 453-462.

14 T.P.J. Linsinger, J. Pauwels, A. Lamberty, H. Schimmel, A.M.H. van der Veen, L. Siekmann, Estimating the Uncertainty of Stability for Matrix CRMs, Fres. J. Anal. Chem. 370 (2001) 183-188

15 International vocabulary of metrology - Basic and general concepts and associated terms (VIM), 3rd Edition, Joint Committee for Guides in Metrology, JCGM 200:2012 html edition, updated on 19th September 2015

16 Certificate of analysis ERM®-AE640 (IRMM-640)

17 G. Audi and A.H. Wapstra, The 1993 atomic mass evaluation, Nucl Phys A565 (1993) 1-65

Annex

Annex A: Results of the homogeneity measurements

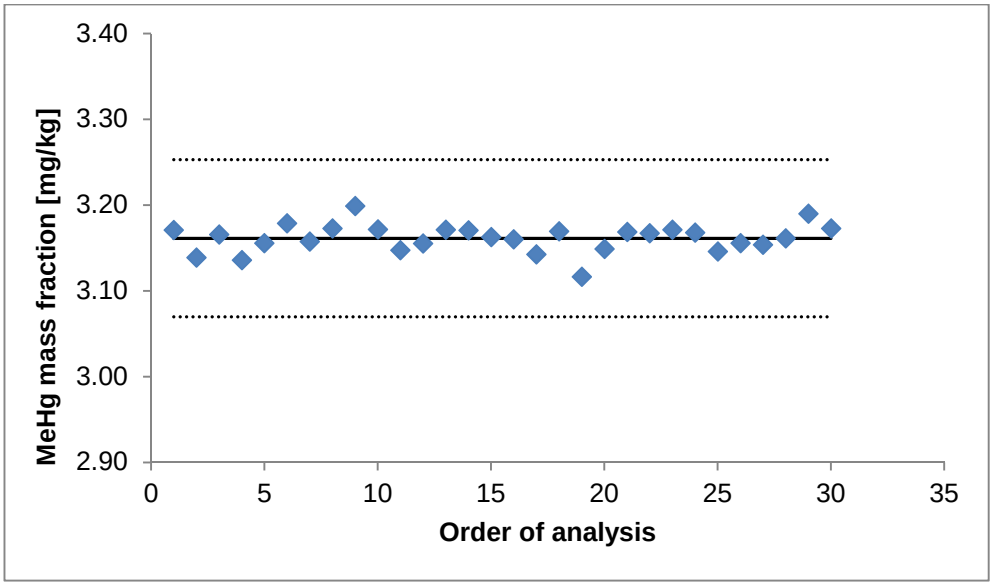

Figure A1: MeHg mass fractions in ERM-AE671 homogeneity study, replicates in function of the analytical sequence. The replicate mass fractions are plotted with the measurement uncertainty (dotted lines) and mean of the mass fractions (solid line). MeHg mass fractions were calculated from measured total Hg and inorganic Hg^{2+} mass fractions. Measurement uncertainty represents the combined measurement uncertainties of total Hg and inorganic Hg^{2+}.

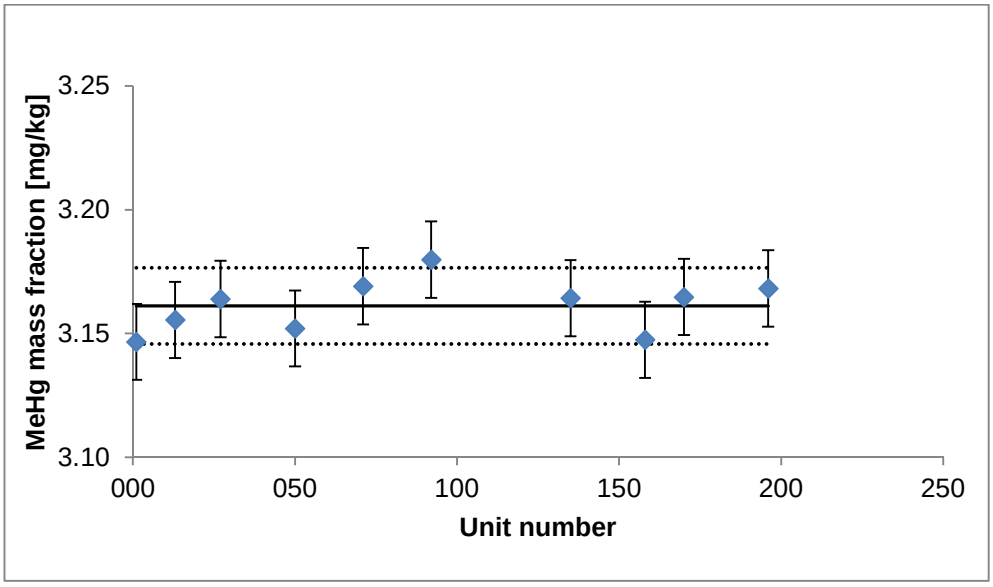

Figure A2: Mean MeHg mass fractions in ERM-AE671 as a function of the unit (ampoule) number. The unit means are plotted with the 95 % CI of the means (s_{wb} from ANOVA for all units). Solid line represents mean of means mass fraction and the dotted lines 95 % CI of the mean of means. MeHg mass fractions were calculated from measured total Hg and inorganic Hg^{2+}.

Table A1: ERM-AE671 MeHg mass fraction results for homogeneity, results in function of the analytical run. MeHg mass fractions were calculated from measured total Hg and inorganic Hg^{2+}.

CRM	Unit number	Replicate number	Total Hg Mass fraction [mg/kg]	Inorganic Hg^{2+} Mass fraction [mg/kg]	MeHg Mass fraction [mg/kg]
ERM-AE671	071	1	3.193	0.0225	3.1705
ERM-AE671	013	3	3.157	0.0184	3.1386
ERM-AE671	071	2	3.188	0.0226	3.1654
ERM-AE671	001	3	3.148	0.0126	3.1354
ERM-AE671	158	2	3.175	0.0199	3.1551
ERM-AE671	135	1	3.201	0.0227	3.1783
ERM-AE671	050	3	3.181	0.0237	3.1573
ERM-AE671	013	2	3.191	0.0185	3.1725
ERM-AE671	092	1	3.217	0.0184	3.1986
ERM-AE671	071	3	3.194	0.0226	3.1714
ERM-AE671	135	2	3.170	0.0228	3.1472
ERM-AE671	170	1	3.180	0.0250	3.1550
ERM-AE671	158	1	3.191	0.0199	3.1711
ERM-AE671	170	2	3.195	0.0248	3.1702
ERM-AE671	001	1	3.175	0.0127	3.1623
ERM-AE671	027	1	3.180	0.0201	3.1599
ERM-AE671	001	2	3.155	0.0127	3.1423
ERM-AE671	170	3	3.194	0.0248	3.1692
ERM-AE671	158	3	3.136	0.0198	3.1162
ERM-AE671	196	1	3.173	0.0246	3.1484
ERM-AE671	092	2	3.187	0.0185	3.1685
ERM-AE671	196	3	3.191	0.0243	3.1667
ERM-AE671	027	2	3.191	0.0200	3.1710
ERM-AE671	135	3	3.190	0.0226	3.1674
ERM-AE671	050	2	3.169	0.0235	3.1455
ERM-AE671	013	1	3.174	0.0186	3.1554
ERM-AE671	050	1	3.177	0.0236	3.1534
ERM-AE671	027	3	3.181	0.0201	3.1609
ERM-AE671	196	2	3.214	0.0245	3.1895
ERM-AE671	092	3	3.191	0.0185	3.1725

Annex C: Results of the short-term stability measurements

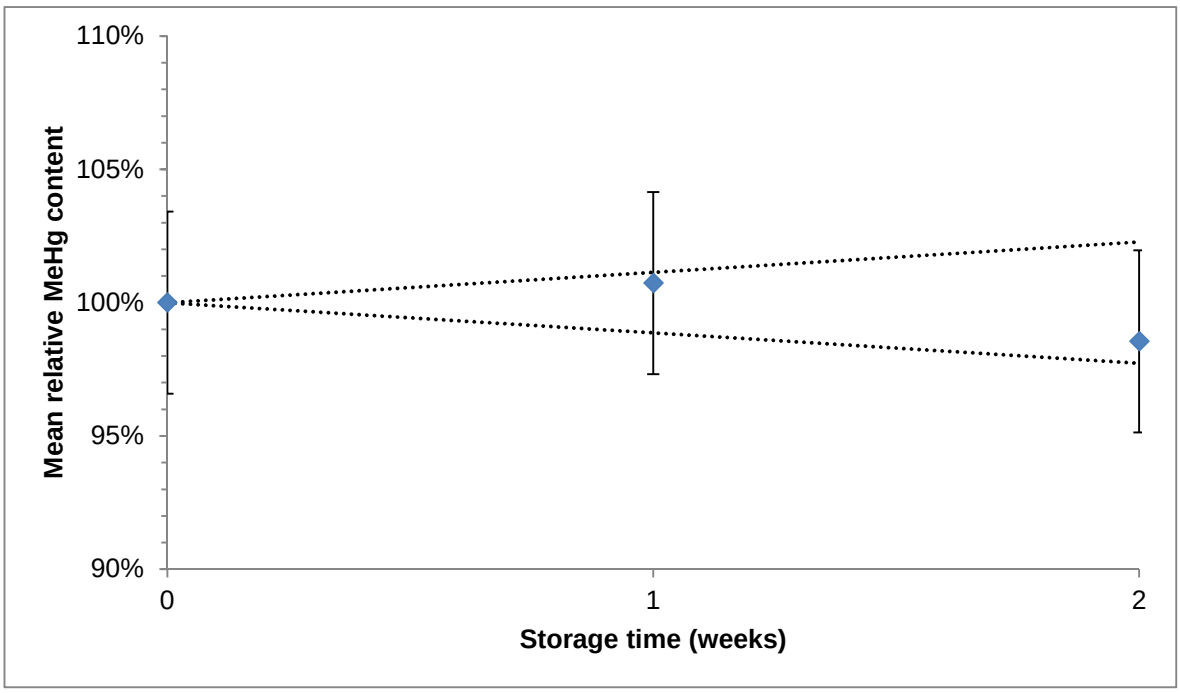

Figure C1: Isochronous STS of ERM-AE671 at 60 °C. Mean relative MeHg content in function of storage time. The mean relative MeHg content is plotted with the 95 % confidence interval of the replicates means.

Table C1: ERM-AE671 MeHg corrected peak area results for short-term stability study. Relative peak area results are calculated with the mean corrected peak area for 0 weeks.

CRM	Unit number	Replicate number	Time [weeks]	Corrected peak area	Relative peak area [%]
ERM-AE671	226	1	0	349.06	99.6
ERM-AE671	226	2	0	337.57	96.3
ERM-AE671	226	3	0	364.62	104.1
ERM-AE671	227	1	1	350.75	100.1
ERM-AE671	227	2	1	347.15	99.1
ERM-AE671	227	3	1	361.10	103.0
ERM-AE671	228	1	2	333.99	95.3
ERM-AE671	228	2	2	350.00	99.9
ERM-AE671	228	3	2	352.00	100.5

Annex D: Results of the long-term stability measurements

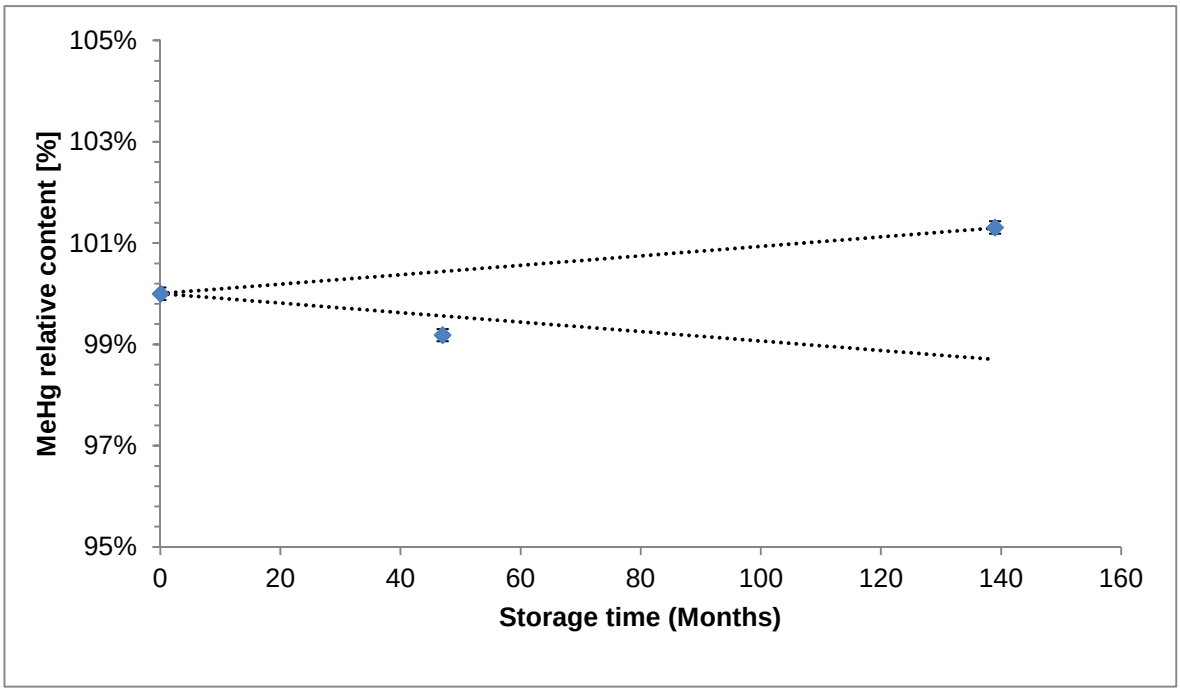

Figure D1: Modified LTS of ERM-AE671 at -20 °C, mean relative MeHg content in function of storage time. The mean relative MeHg contents are plotted with the 95 % CI of the sample (unit) means.

Annex E: Summary of methods used in the characterisation study

Table E1: Method information used in the ERM-AE671 process control.

Laboratory / Method code	Parameter	Sample preparation	Technique	Calibration	Instrumentation
L00	Hg total mass fraction	24 h in conc. HNO_3, digestion at ambient temperature	ICP-MS	ID with ERM-AE671 spike	ELAN 6000
L00	Inorganic Hg^{2+} mass fraction	Organic phase extraction and conversion to butyl- derivatives	GC-ICP-MS	External calibration with ERM-AE671	Agilent N6890 GC coupled with ELAN 6000 ICP-MS
L00	MeHg mass fraction	Organic phase extraction and conversion to butyl- derivatives	GC-ICP-MS	External calibration with standards prepared from Merck MeHgCl (> 98 %)	Agilent N6890 GC coupled with ELAN 6000 ICP-MS

Table E2: Method information used in the ERM-AE671 verification study.

Laboratory / Method code	Parameter	Sample preparation	Technique	Calibration	Instrumentation
L01	Hg total mass fraction	HNO_3 digestion in Ultraclave	ICP-SFMS	Reverse ID with ERM-AE639 spike	Element XR
L01	Inorganic Hg^{2+} mass fraction	Dilution, ion-exchange column separation	ICP-SFMS	External calibration with UltraScientific iHg 1000 mg/L, ICP-080 lot P00139	Element XR
L01	MeHg mass fraction	Dilution, ethylation, purge and trap on Tenax, thermal desorption	GC-ICP-SFMS	Reverse ID with Spike from Alfa Aesar 1000 mg/L MeHgCl, abundances from IUPAC (1998)	Element 2
L01	Isotopic abundance / total Hg	HNO_3 digestion in Ultraclave	ICP-MCMS	Bracketing standards, UltraScientific iHg 1000 mg/L, ICP-080 lot P00139	Neptune
L01	Isotopic abundance / Hg^{2+} inorganic	Dilution, ion-exchange column separation	ICP-SFMS	Bracketing standards, UltraScientific iHg 1000 mg/L, ICP-080 lot P00139	Element XR
L01	Isotopic abundance MeHg	Dilution, ion-exchange column separation	ICP-SFMS	Bracketing standards, UltraScientific iHg 1000 mg/L, ICP-080 lot P00139	Element XR

Annex F: Results of the gravimetric dilution, verification measurements and process control

Table F1: MeHg amount content. The table shows MeHg amount content as calculated by multiplication of the certified value of ERM-AE670 with the gravimetric dilution factor.

Laboratory code	Mean [mol/g]	Expanded uncertainty [mol/g]
L00 (2007)	$15.47 \cdot 10^{-9}$	$0.55 \cdot 10^{-9}$

Table F2: Total Hg amount content of ERM-AE671. The table shows total Hg amount content as calculated by multiplication of the total Hg content for ERM-AE670 with the gravimetric dilution factor.

Laboratory code	Mean [mol/g]	Expanded uncertainty [mol/g]
L00 (2007)	$15.767 \cdot 10^{-9}$	$0.097 \cdot 10^{-9}$

Table F3: Inorganic Hg^{2+} amount content. Results used for verification of MeHg amount content

Laboratory code	Ampoule 001 [mol/g]	Ampoule 013 [mol/g]	Ampoule 027 [mol/g]	Ampoule 050 [mol/g]	Ampoule 071 [mol/g]	Mean [mol/g]	Expanded uncertainty [mol/g]
	Ampoule 092 [mol/g]	Ampoule 135 [mol/g]	Ampoule 158 [mol/g]	Ampoule 170 [mol/g]	Ampoule 196 [mol/g]		
L01 (2015)	$63 \cdot 10^{-12}$	$92 \cdot 10^{-12}$	$99 \cdot 10^{-12}$	$117 \cdot 10^{-12}$	$112 \cdot 10^{-12}$	$103 \cdot 10^{-12}$	$13 \cdot 10^{-12}$
	$92 \cdot 10^{-12}$	$112 \cdot 10^{-12}$	$98 \cdot 10^{-12}$	$123 \cdot 10^{-12}$	$121 \cdot 10^{-12}$		

Table F4: Total Hg amount content. The table shows measured total Hg values used for confirmation of the gravimetric value.

Laboratory code	Ampoule 001 [mol/g]	Ampoule 013 [mol/g]	Ampoule 027 [mol/g]	Ampoule 050 [mol/g]	Ampoule 071 [mol/g]	Mean [mol/g]	Expanded uncertainty [mol/g]
	Ampoule 092 [mol/g]	Ampoule 135 [mol/g]	Ampoule 158 [mol/g]	Ampoule 170 [mol/g]	Ampoule 196 [mol/g]		
L01 (2015)	$15.65 \cdot 10^{-9}$	$15.72 \cdot 10^{-9}$	$15.77 \cdot 10^{-9}$	$15.73 \cdot 10^{-9}$	$15.81 \cdot 10^{-9}$	$15.76 \cdot 10^{-9}$	$0.45 \cdot 10^{-9}$
	$15.84 \cdot 10^{-9}$	$15.78 \cdot 10^{-9}$	$15.69 \cdot 10^{-9}$	$15.80 \cdot 10^{-9}$	$15.81 \cdot 10^{-9}$		

Table F5: MeHg amount content. The table shows calculated MeHg amount content as calculated from total Hg obtained by gravimetric dilution and measured inorganic Hg^{2+} amount contents and directly measured MeHg amount content.

Laboratory code	Ampoule 001 [mol/g]	Ampoule 013 [mol/g]	Ampoule 027 [mol/g]	Ampoule 050 [mol/g]	Ampoule 071 [mol/g]	Mean [mol/g]	Expanded uncertainty [mol/g]
	Ampoule 092 [mol/g]	Ampoule 135 [mol/g]	Ampoule 158 [mol/g]	Ampoule 170 [mol/g]	Ampoule 196 [mol/g]		
Results used for verification of certified MeHg amount content - calculated value							

L01	$15.70 \cdot 10^{-9}$ $15.68 \cdot 10^{-9}$	$15.68 \cdot 10^{-9}$ $15.65 \cdot 10^{-9}$	$15.67 \cdot 10^{-9}$ $15.67 \cdot 10^{-9}$	$15.65 \cdot 10^{-9}$ $15.64 \cdot 10^{-9}$	$15.66 \cdot 10^{-9}$ $15.65 \cdot 10^{-9}$	$15.66 \cdot 10^{-9}$	$0.10 \cdot 10^{-9}$ 1)
Results used for verification of certified MeHg amount content - measured value							
L01	$15.81 \cdot 10^{-9}$ $16.25 \cdot 10^{-9}$	$16.53 \cdot 10^{-9}$ $15.71 \cdot 10^{-9}$	$16.15 \cdot 10^{-9}$ $16.10 \cdot 10^{-9}$	$15.95 \cdot 10^{-9}$ $16.34 \cdot 10^{-9}$	$15.81 \cdot 10^{-9}$ $15.98 \cdot 10^{-9}$	$16.09 \cdot 10^{-9}$	$0.74 \cdot 10^{-9}$ 2)

1) Combined uncertainty of $u_{meas}(Hg^{2+})$ and $u_{grav}(^{total}Hg)$
2) u_{meas}(MeHg)

Table F6: Measured total Hg mass fractions in ampoules filled with diluent solution. 5 ampoules were tested from the morning and 5 ampoules from the afternoon filling session.

Ampoules	n	^{200}Hg mass fraction kg (Hg)·kg^{-1}	1 SD	^{202}Hg mass fraction kg (Hg)·kg^{-1}	1 SD
morning	5	$1.4 \cdot 10^{-12}$	14 %	$1.2 \cdot 10^{-12}$	17 %
afternoon	5	$1.2 \cdot 10^{-12}$	10 %	$4.6 \cdot 10^{-12}$	10 %

Table F7: The table shows a comparison of Hg isotopic ratios between ERM-AE670 certified values and isotopic ratios measured for verification in ERM-AE671 in 2015.

Hg isotopic ratios	**196/202**	**198/202**	**199/202**	**200/202**	**201/202**	**204/202**
Certified Hg in MeHg (ERM-AE670)	0.000018	0.000623	0.001603	0.0055	0.01335	0.0026
Measured ^{total}Hg (ERM-AE671)	0.000017	0.000650	0.001632	0.0056	0.01352	0.0026
Measured Hg in MeHg (ERM-AE671)	0.000029	0.000664	0.001607	0.0056	0.01360	0.0027

Table F8: The table shows a comparison of measured inorganic Hg^{2+} isotopic ratios for verification measurements in 2015 with ERM-AE670 and ERM-AE671 measurements made in 2003 and 2007

Hg isotopic ratios	**196/202**	**198/202**	**199/202**	**200/202**	**201/202**	**204/202**
Measured inHg^{2+} (ERM-AE670)	0.000032	0.002230	0.004202	0.0089	0.01525	0.0036
Measured inHg^{2+} (ERM-AE671) -2007	0.000313	0.001101	0.000346	0.0071	0.01408	0.0033
Measured inHg^{2+} (ERM-AE671) -2015	0.000497	0.002212	0.003855	0.0087	0.01536	0.0037

Table F9: The table shows a comparison between certified ERM-AE670 and measured ERM-AE671 isotope abundance fractions

	ERM-AE670 Hg in the form of MeHg		ERM-AE671 measured total Hg		ERM-AE671 measured Hg in the form of MeHg		ERM-AE671 measured Hg in the form of inorganic Hg^{2+}	
Isotope	Isotope abundance fraction	U (k=2)	Isotope abundance fraction	U (k=2)	Isotope abundance fraction	U (k=2)	Isotope abundance fraction	U (k=2)
196Hg	0.0018	0.0013	0.0016	0.0008	0.0028	0.0046	0.048	0.056
198Hg	0.0609	0.0049	0.0635	0.0056	0.0648	0.0076	0.214	0.126
199Hg	0.15659	0.0009	0.1594	0.0077	0.1569	0.0068	0.3727	0.177
200Hg	0.537	0.021	0.543	0.012	0.548	0.030	0.839	0.330
201Hg	1.304	0.051	1.320	0.018	1.328	0.037	1.485	0.190
202Hg	97.685	0.057	97.663	0.035	97.640	0.089	96.682	1.095
204Hg	0.254	0.016	0.250	0.012	0.259	0.0092	0.359	0.183

European Commission

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